

**Use of inhibitors of enzymes having activities of amino peptidase N or/and dipeptidyl peptidase IV and of pharmaceutical preparations thereof for a therapy and prevention of dermatological diseases with sebaceous hyperproliferation and modified conditions of differentiation**

**Description**

The invention describes the inhibition of the DNA synthesis necessary for the proliferation of sebaceous cells (sebocytes) by the effect of inhibitors of amino peptidase N (APN; E.C. 3.4.11.2, CD13) or/and of dipeptidyl peptidase IV (DP IV; E.C. 3.4.14.5, CD26) as the result of the separate, of the simultaneous or, of the, with respect to the time, immediately successive application of respective specific inhibitors of these enzymes or of inhibitors of enzymes having a similar substrate specificity (APN- or/and DP IV-analogous enzyme activity) on the basis of amino acid derivatives, peptides or peptide derivatives by which the proliferation (DNA synthesis) of sebocytes is suppressed.

A number of dermatological diseases are associated with sebaceous or seborrheic hyperproliferation and modified conditions of differentiation. Among them are both benign follicular hyperproliferation conditions (acne, acneiform follicular reactions, steatocystoma multiplex, naevi of sebaceous glands, senile sebaceous gland hypertrophy, seborrhea of the skin and of the hair) and malign follicular hyperproliferation conditions (mixed tumors, sebaceomes, sebaceous gland tumors, sebaceous gland CA).

Peptidases, such as dipeptidyl peptidase IV and amino peptidase N or similarly effecting enzymes are of particular interest for the regulation or/and modulation of interactions between cells, since they are, inter alia, localized in the plasma membrane of the cells as ectoenzymes, incur interactions with other extracellular structures, activate or inactivate peptidergic messenger substances by enzyme-catalyzed hydrolysis, and, are thereby important for a cell-to-cell communication. [Yaron A, et al.: Proline-dependent structural and biological properties of peptides and proteins. Crit Rev Biochem Mol Biol 1993; 28 : 31 - 81;

Vanhoof G, et al.: Proline motifs in peptides and their biological processing. *FASEB J* 1995; 9: 736 - 744].

It was shown that membrane-allocated peptidases like DP IV or APN play a key role in the process of the activation and clonal expansion of immune cells, in particular of T-lymphocytes. [Fleischer B: CD 26 a surface protease involved in T-cell activation. *Immunology Today* 1994; 15: 180 - 184; Lendeckel U et al.: Role of alanyl aminopeptidase in growth and function of human T cells. *International Journal of Molecular Medicine* 1999; 4: 17 - 27; Riemann D et al.: CD13 - not just a marker in leukemia typing. *Immunology Today* 1999; 20: 83 - 88]. Different functions of mitogene-stimulated mononuclear cells (MNZ) or of enriched T lymphocytes such as DNA-synthesis, production and secretion of immuno stimulating cytokines (IL-2, IL-6, IL-12, IFN- $\gamma$ ) and helper functions for B-cells (IgG synthesis and IgM synthesis) may be inhibited in the presence of specific inhibitors of DP IV or of APN [Schön E, et al.: The dipeptidyl peptidase IV, a membrane enzyme involved in the proliferation of T lymphocytes. *Biomed. Biochim. Acta* 1985; 2: K9-K15; Schön E, et al.: The role of dipeptidyl peptidase IV in human T lymphocyte activation. Inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and immunoglobulin synthesis in vitro. *Eur. J. Immunol.* 1987; 17: 1821-1826; Reinhold D, et al.: Inhibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor  $\beta$ 1 in PWM-stimulated PBMNC and T cells. *Immunology* 1997; 91: 354-360; Lendeckel U, et al.: Induction of the membrane alanyl aminopeptidase gene and surface expression in human T-cells by mitogenic activation. *Biochem. J.* 1996; 319: 817-823; Kähne T, et al.: Dipeptidyl peptidase IV: A cell surface peptidase involved in regulating T cell growth (Review). *Int. J. Mol. Med.* 1999; 4: 3-15; Lendeckel U, et al.: Role of alanyl aminopeptidase in growth and function of human T cells (Review). *Int. J. Mol. Med.* 1999; 4: 17-27]. It is already known that the treatment of autoimmune diseases and transplant rejection is possible by an inhibition of dipeptidyl peptidase IV localized on immune cells by means of synthetic inhibitors (e.g. EP764 151 A1, WO 09529691, EP731789, EP528 858).

The invention is based on the surprising finding that the single or simultaneous effect of inhibitors of the dipeptidyl peptidase IV/CD26 or/and inhibitors of the amino peptidase N/CD13 or of inhibitors of enzymes having a similar substrate specificity (APN- or/and DP IV-analogous enzyme activity), expressed on or in sebaceous cells (sebocytes) inhibits the proliferation (DNA synthesis) of these cells.

Our invention shows that, for therapy and for prevention of dermatological diseases with sebaceous hyperproliferation and modified conditions of differentiation (benign follicular hyperproliferation conditions like acne, acneiform follicular reactions, steatocystoma multiplex, naevi of sebaceous glands, senile sebaceous gland hypertrophy, seborrhea of the skin and of the hair as well as malign follicular hyperproliferation conditions like mixed tumors, sebaceomas, sebaceous gland tumors, sebaceous gland CA) for the generation of which the proliferation of sebocytes has a central importance, the single or simultaneous application of inhibitors of DP IV and of APN or of inhibitors of enzymes having the same substrate specificity (APN- or/and DP IV-analogous enzyme activity) or of corresponding preparations and application forms thereof is suitable.

In detail, the invention is based on the findings that the DNA synthesis of sebaceous cells (sebocytes) is significantly inhibited by the administration of inhibitors of dipeptidyl peptidase IV or/and of inhibitors of amino peptidase N.

Up to now, the above-mentioned diseases are treated topically or/and systemically by administering antibiotics or/and antiproliferative and differentiating substances (antiandrogens, 13-cis-retinoic acid and others). In the systemical treatment in particular, undesired side effects often appear. These are inter alia teratogenicity, lipid metabolic disorders, psychoreactive phenomena, gastrointestinal disorders as well as muco-cutaneous irritative reactions.

The use of DP IV or/and APN inhibitors would represent a completely new, presumably very effective, possibly cost-effective therapy form and a valuable alternative component of existing therapy concepts of the above-referenced diseases.

The inhibitors of dipetidyl peptidase IV or/and the inhibitors of amino peptidase N or inhibitors of enzymes having the same substrate specificity (APN-analogous or/and DP IV-analogous enzyme activity) applied according to the invention may be administered in pharmaceutically applicable formulation complexes as inhibitors, substrates, pseudo substrates, inhibitorily effective peptides and peptide derivatives as well as antibodies to those enzymes.

Preferred effectors for DP IV, are for example, Xaa-Pro-dipeptides, corresponding derivatives, preferably dipeptide phosphonic acid diaryl esters and their salts, Xaa-Xaa-(Trp)-Pro-(Xaa)<sub>n</sub> peptides (n = 0 to 10), corresponding derivatives and their salts or amino acid (Xaa)-amides, corresponding derivatives and their salts, wherein Xaa is an  $\alpha$ -amino acid/imino acid or an  $\alpha$ -amino acid derivative/imino acid derivative, preferably N<sup>ε</sup>-4-nitrobenzyl oxycarbonyl-L-lysine, L-proline, L-tryptophane, L-isoleucine, L-valine, and cyclic amines, e. g. pyrrolidine, piperidine, thiazolidine, and their derivatives as amide structure. Such compounds and their preparation were described in an earlier patent (K. Neubert et al. DD296075A5). Preferred inhibitors for the alanyl amino peptidase are bestatin (ubenimex), actinonin, probestin, phebestin, RB3014 or leuhistin.

The inhibitors are administered simultaneously with known carrier substances. On the one hand, the administration occurs as a topical application in the form of, for example, cremes, ointments, pastes, gels, solutions, sprays, liposomes and nanosomes, agitated mixtures, hydrocolloid dressings, plasters and similar novel carrier substrates, jet injections or other dermatological bases/vehicles, including instillative applications, and on the other hand, as a systemic application for oral, transdermal, intravenous, subcutaneous, intracutaneous, intramuscular use in suitable formulations or in a suitable galenic form.

### Example 1

#### **Inhibition of the DNA synthesis of the immortalized human sebaceous cell line SZ95 by the incubation with synthetic inhibitors of DP IV or/and APN**

Our investigations show that the DNA synthesis of the immortalized human sebaceous cell line SZ95 (Zouboulis, C.C. et al: Establishment and characterization of an immortalized human sebaceous gland cell line (SZ95). J. Invest. Dermatol. 1999, 113: 1011 – 1020) is inhibited by the administration of inhibitors of the DP IV (Lys[Z(NO<sub>2</sub>)]-thiazolidide or/and of the APN (actinonin) in a dose-dependent manner.

The human sebaceous cell line SZ95, which is accepted as a cell model for acne, expresses strongly DP IV and APN (Figure 1). The enzyme activity of the DP IV of vital cells amounts to  $38 \pm 18$  pkat/10<sup>6</sup> cells, and that of the APN amounts to  $262 \pm 58$  pkat/10<sup>6</sup> cells (n = 3). Accordingly, the mRNA of APN and DP IV is detectable on these cells (Figure 2).

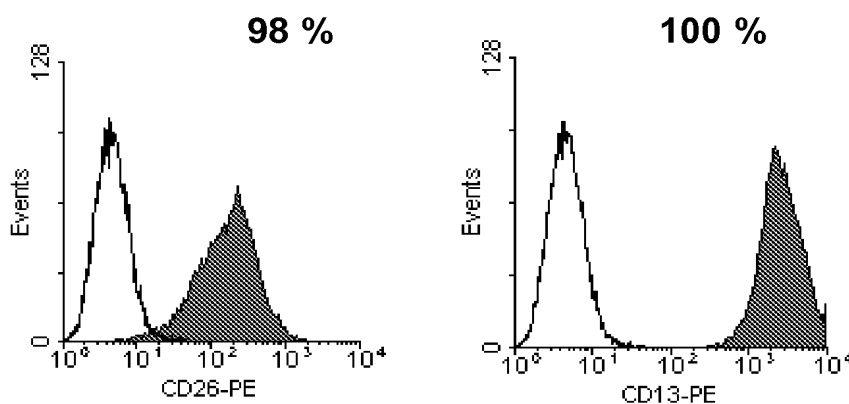


Figure 1: Cytometric flow rate verification of the expression of DP IV (CD26) and APN (CD13) on SZ95 cells

SZ95 cells were incubated for 48 hours with the above-mentioned inhibitors and subsequently the DNA synthesis was determined by the measurement of the <sup>3</sup>[H]-Thymidine

incorporation as described in Reinhold et al. (Reinhold, D et al.: Inhibitors of dipeptidyl peptidase IV induce secretion of transforming growth factor  $\beta$ 1 in PWM-stimulated PBMNC and T-cells. Immunology, 1997, 91: 354 – 360). Figure 2 shows the inhibition of the DNA synthesis depending on the dose.

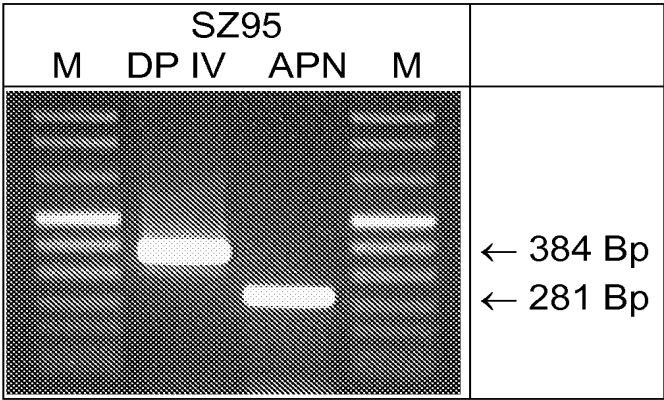


Figure 2: Verification of the mRNA expression of DP IV (CD26) and APN (CD13) on SZ95 cells via RT-PCR

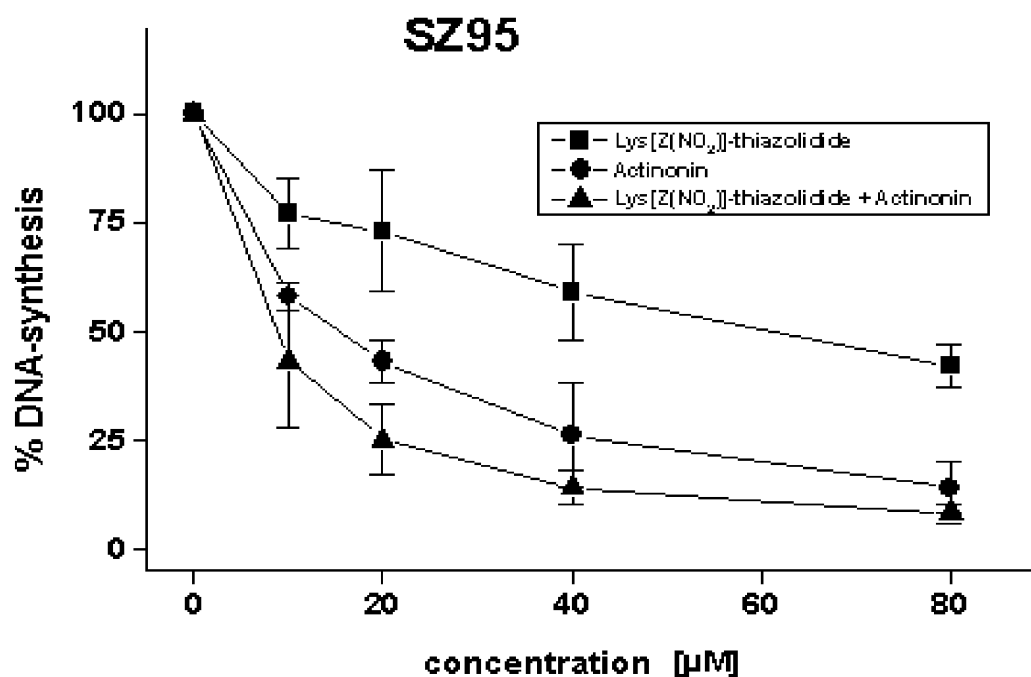


Figure 3: Effect of inhibitors of the DP IV (Lys[Z(NO<sub>2</sub>)]-thiazolidide) and of the amino peptidase N (actinonin) on the DNA synthesis of human SZ95 sebaceous cells depending on the dose.

The cells were incubated for 48 hours with the mentioned concentrations of the inhibitors. Subsequently <sup>3</sup>[H]-Methyl-thymidine was added to the culture medium and after additional 6 hours the amount of <sup>3</sup>[H]-Thymidine incorporated in the DNA was measured.